ELECTRO- AND MAGNETOBIOLOGY, 20(3), 397–413 (2001)

EFFECTS OF SINUSOIDAL MAGNETIC FIELD ON ADHERENCE INHIBITION OF LEUKOCYTES

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ABSTRACT

Response of leukocytes to exposure to an external magnetic field with frequency 50 Hz and sinusoidal waveform was investigated in vitro using the leukocyte adherence inhibition (LAI) assay developed as a measure of cell-mediated immunity. Leukocytes taken from healthy humans adhere, but their adherence decreases after 1 hr of exposure to the magnetic field with magnetic induction of 1 and 10 mT. The majority of leukocytes taken from cancer patients before any medical treatment do not adhere, and exposure to the magnetic field increases adherence. Correlation between the LAI assay results and the cell-mediated immunity suggests an effect of magnetic fields on leukocyte immune function in humans.

Key Words: Biological effects of magnetic field; Cell adherence inhibition; Cell-mediated immunity

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INTRODUCTION

Cell-mediated immunity is understood to correlate with the adherence properties of leukocytes (from peripheral blood). The leukocyte adherence inhibition (LAI) assay is an in vitro quantitative technique that has been widely used to study and monitor cell-mediated immunity (1–11). The LAI assay is based on observation of in vitro adherence of leukocytes to glass surfaces or some plastic materials in the presence or absence of antigens.

Specific cell-mediated immune responses are made by T lymphocytes. There are two main classes of T cells: helper T cells and cytotoxic T cells. Helper T cells regulate immune responses. Cytotoxic T cells are involved in defense against infection and kill infected cells. Helper T cells help to activate immune responses by secreting local mediators—cytokines. The helper T cell starts its activity after recognizing foreign antigen in association with class II major histocompatibily complex (MHC) protein on the surface of an antigen-presenting cell. Two simultaneous signals must be present for activation of a helper T cell. Signal 1 is provided by interaction of a foreign peptide bound to a class II MHC molecule on the surface of the antigen-presenting cell (an important role in binding and creation of the signal transmission pathway has T-cell receptor (TCR) protein on the surface of the helper T cell). Signal 2 is provided either by a chemical signal (e.g., by interleukin-1) secreted by the antigen-presenting cell or by the signaling molecule B7, which binds to a coreceptor protein CD28 on the surface of the helper T cell. All of these mechanisms are necessary for correct function of cell-mediated immmunity. It is evident that helper T cells (which are also called CD4 lymphocytes) stimulating the immune response of other white blood cells have a fundamental role in the immune system. In T cell trafficking, interactions with the surrounding extracellular matrix play an important role. Recirculation between blood vessels and the lymphoid tissues is dependent on adhesion mediated by selectins and integrins (12,13).

Numerous experimental LAI assays have been designed for quantitative assessment of cell-mediated immunity of leukocytes, in particular of tumor-specific cell-mediated immunity (5–11,14). Leukocytes taken from immunized individuals have diminished adherence if corresponding antigen is present in the suspension. The adherence process is described in references 15 and 16. Experimental results have shown a gap of about 10 nm between the adherent cell and the substrate (17), and Curtis has explained the adherence of cells on the basis of electromagnetic interaction (attractive London dispersion forces and repulsive electrostatic forces) (18). The long-range London force arises from fluctuating electric dipoles due to atomic and molecular vibration. These fluctuations give rise to a fluctuating electromagnetic field and to electromagnetic interactions between the dipoles of neighboring atoms and molecules, and a force of attraction develops. The long-range adherence mechanism may depend on the electromagnetic field generated by excited vibrations of structures with electric dipole moment (e.g., of the Froeh-

lich type) (19–22). The mechanism of these forces is similar to that of London dispersion forces, but the excited vibrations are far from thermodynamic equilibrium. Maroudas (23,24) revealed the dependence of adherence and spreading of cells upon a substrate on the surface charge of the substrate. This theory of the long-range mechanism is supported by observation of interaction forces between leukocytes and of the formation of clusters (25). In addition to London forces, electrostatic double-layer forces are present between charged surfaces in electrolyte solutions. Counterions to the surface form a diffusion layer at the charged surface. When two charged surfaces are so close together that their diffuse layers overlap, an electrostatic double-layer interaction arises (26). Therefore, adherence of cells includes two different mechanisms: long range and short range (27). The short-range mechanism is a domain of electrostatic forces and chemical binding. It is not quite clear whether integrins (β_2) or selectins, which enable white blood cells to attach to and to cross the endothelial lining of blood vessels, are involved in adherence to solid-state surfaces. Adherence seems to be highly specific (28).

Koppi et al. (4) found that supernatants contained soluble leukocyte adherence inhibition factor (LAIF) produced by T cells. The LAIF reduced the adherence of leukocytes but its mechanism of action was not known.

The assumption that the oncogenic process can alter leukocyte-mediated immunity led to attempts to use the LAI assay for detection of malignacies in humans (14). The LAI assay enables us to reliably disclose with increased sensitivity and specificity adherence inhibition of leukocytes taken from cancer patients (19–21).

Chemical as well as physical agents can change adherence properties of leukocytes. Antigen alters adherence after it binds to the corresponding receptor in the cell membrane. After binding, a sequence of successive signaling processes is triggered, resulting in changed adherence of the leukocyte cell. Magnetic field is one physical agent that can alter behavior of the leukocytes. First, we will present an overview of a variety of effects in living cells and in living matter generally induced by magnetic field with power supply frequencies (i.e., 50 or 60 Hz). Increased enzyme activities of protein kinase C, cytochrome oxydase, Na,K ATPase, and ornithine decarboxylase have been observed (29–33). For instance, cytochrome oxidase activity is changed by a weak magnetic field, and the effect depends on frequency of the field. The rate constant is increased by about 30% at fields below 3 μT, and by a factor of 2–3 at fields between 6 and 10 μT (30). The greatest effects appear at frequencies of 500–1000 Hz (31). Hydrophilic pores can be formed in the plasma membrane by magnetic pulses greater than about 10^{-3} T (34).

A magnetic field acts on opening or closing of ion channels in plasma membranes, e.g., on calcium channels (32). Ca^{2+} ion concentration in cells is changed by a magnetic field at 50 Hz and 100 μT (35). Pulsed magnetic field (0.07–1.6 T at intervals of 3 sec) causes inhibition of K^+ influx into the cell (36). Exposure to magnetic field simulates biosynthetic stress, and elicits expression of the heat

shock protein Hsp70 (37–39). For instance, 20 min of exposure produced a maximum of the heat shock element-binding at 20 min following exposure (39). Cells respond to magnetic field as to an environmental stress (40). Tenforde (41) presented a hypothesis that exposure to external fields initiates electrochemical events within the cell membrane. Protein strands that protrude from within the cell membrane might form electrochemical ''antennae'' (42). On the other hand, magnetic fields penetrate the cell, and can interact inside the cell too (40).

Exposure of mice to magnetic fields (50 Hz, 2 mT) has been shown to induce a cytotoxic effect in their central and peripheral lymphoid tissues (43). Magnetic fields affect immune system responses to mitogens and antigens strongly (44). Cytokine production by human peripheral blood mononuclear cells is altered after exposure to sinusoidal magnetic field from 50 μT to 2 mT (45). Increased production of growth factors and cytokines by peripheral blood mononuclear cells after exposure to a pulsed magnetic field of 3 mT peak is reported in reference 46. After exposure to a magnetic field of 50 Hz, 2 mT (rms), human lymphoid cells exhibit morphological effects on the organization of the plasma membrane, decrease in cell roughness (possibly related to a loss of microvilli), and an altered pattern of protein phosphorylation (47). T lymphocytes' CD3 antibody release was significantly increased in a magnetic field (48). Mehta et al. reported increased proliferation of T and B cells in reference 49 (after exposure to an oscillating magnetic field), but no change in proliferation ratio of peripheral blood mononuclear cells was observed in reference 50 (after exposure to static and oscillating magnetic fields). Milani et al. exposed lymphocytes to a pulsating 50 Hz magnetic field whose intensity was comparable with that of the Earth's magnetic field (51). After exposure for 72 hr, damage of the cell membranes in irradiated lymphocytes was observed. The morphology of irradiated cells was dramatically different from controls. The nuclei were frequently vesicular, devoid of chromatin, with cytoplasm not circumscribed, and embedded in a finely granular proteinaceous matrix.

We will examine the effects of a sinusoidal magnetic field with a frequency 50 Hz on cell-mediated immunity of leukocytes by the LAI assay. The experimental results contribute to understanding of the effects of power line magnetic fields on humans at the cellular and subcellular levels. Preliminary experimental results of the effects of magnetic field on T lymphocytes have already been published in reference 27.

MATERIALS AND METHODS

T cells were separated from venous blood of healthy humans ($N = 25$) and cancer patients ($N = 24$) (otorhinolaryngological tumors—ca *pharyngis*). Histopathological verification of the cancer diagnosis was made. We also measured T lymphocytes taken from patients with ca *linguae* (radicis) and *tonsilae*, but these small groups were not included in the results presented in this paper.

Preparation of T Cells

Eight milliliters of venous blood was taken from the patient and transferred to a test tube with 500 U of heparin, and incubated in a water bath for 30 min at 37°C. A Pasteur pipette was used to transfer the plasma rich in white blood cells into a new test tube. The contents were diluted with saline solution or phosphate buffered saline (PBS), minimum essential medium (MEM), medium to its original volume and centrifuged at 1800 rpm. The supernatant was poured off and the sediment was layered with 1 ml of distilled water, bubbled through a Pasteur pipette, and lysis of erythrocytes was achieved. Thereafter, dilution to volume with some of the above-mentioned solutions according to an option was done. Thus, the lysis of erythrocytes was stopped. The contents were centrifuged once again for 10 min at 1800 rpm. The rinsing solution was poured off, the sediment of white blood cells was diluted with a selected medium, filtered through nylon absorbent wool (resulting in enrichment of the T cells), and diluted again so as to make available 4×10^6 cells per ml for the test, as counted in a Bürker chamber.

For purification of T cells, we used a 5-cm^3 syringe filled with nylon absorbent wool as a filtration column. The pool passing through the column contained about 92% of T cells and 8% of other cells (28).

Preparation of the Immunoactive Fraction from Malignant Tumor (Specific Antigen)

The biological material was taken during surgery, in cooperation with the histopathologist, and placed into a sterile vessel in dry ice. After the material was defrosted, fat and/or necrotic parts were removed. Homogenization was then carried out, and the homogenate was cryolyzed three times at −60 and +40°C. Thereafter, it was centrifuged at $3.5 \times 10^3 \times g$ at +4°C for 40 min. The supernatant was subject to centrifugation again at $10^5 \times g$ at +40°C for 60 min. The supernatant, which contains proteins, nucleoproteins, nucleic acids, and low molecular weight components was extracted with phenol for 15 min at ambient temperature at a volume ratio of 1:1 in the presence of 1% sodium dodecylsulfate and 0.01% of 8-hydroxyquinoline, and pH was adjusted to 7–7.5.

By means of a centrifuge at $3 \times 10^3 \times g$, the aqueous phase was separated and shaken again with phenol. The aqueous phase, containing a mixture of cytoplasmatic ribonucleic acids, was further separated on a column filled with oligo–dT-cellulose (cellulose with bound deoxythimidine oligonucleotides) having affinity for polyadenylated remnants characteristic of information ribonucleic acids. The polyadenylated ribonucleic acids captured on the column were rinsed with water, and the aqueous eluate was further purified with high-pressure gel chromatography. The column was filled with Separon-Hema-300 glc® gel, which is a

suspension copolymer of 2-hydroxyethyl-methacrylate with ethylenedimethacrylate with an exclusion limit of 3×10^5 kDa.

Detection was carried out by means of an R 043 differential refractometer and a UV flow-through spectrophotometer with variable wavelengths. Saline solution was used as a mobile phase. Immunologically active and organ-specific antigens were obtained in the 25–50 cm^3 fraction of the eluate evaluated at the wavelength of 340 nm.

Preparation of Immunologically Functional Fraction from the Inbred Strain Mice Serum (Nonspecific Antigen–Lactate Dehydrogenase Virus [LDV] Antigen)

The immunologically functional fraction was obtained from the serum of inbred laboratory mouse strains, e.g., C3H infected with the virus (LDV) enhancing the lactate dehydrogenase isoenzyme. Plasma obtained from blood treated with heparin was subjected to at least doubled centrifugation for 20–30 min at $3–3.5 \times 10^3 \times g$, and the supernatant taken was clarified for 1–2 min at $9–11 \times 10^3 \times g$. The supernatant was subjected to further centrifugation for 50–70 min at $50–100 \times 10^3 \times g$, and the sediment was suspended in buffer with a pH of 7–7.4 consisting of tris-(hydroxymethyl)-aminomethane-NaCl [Tris-NaCl] and the disodium salt of ethylenediaminetetraacetic acid (EDTA) in a minimum volume of at most 1 ml. Equilibrium centrifugation in a saccharose density gradient separated the constituents, and the area corresponding to 32–38% saccharose with the maximum at 35% saccharose. This fraction was taken off and, after dilution with the above-mentioned buffer at a volume ratio of 1:0.5 to 1.5, was subjected to centrifugation at $50–100 \times 10^3 \times g$, for 50–70 min. Manipulation of blood plasma until the last sediment was obtained was carried out at 2–6°C. The sediment obtained was dissolved in 1.5–3.0 ml of saline solution and used for high-pressure gel chromatography. The fractions corresponding to molecular masses of 3×10^5 to 1.5×10^4 kDa were immunologically active. The equilibrium centrifugation was carried out for a period of 180–210 min at $150–180 \times 10^3 \times g$. Separon Hema-300 glc®, which is a suspension copolymer of 2-hydroxyethyl-methacrylate with ethylenedimethacrylate with an exclusion limit of the column of 3×10^5 kDa, was used as the stationary phase for the high-pressure gel chromatography. Gel particle size was 32–40 μm. A differential refractometer and a UV spectrophotometer with variable wavelength were used for detection. Saline solution was used as a mobile phase, and the active fraction was found using a wavelength of 340 nm.

Measurement

The sinusoidal magnetic field was generated inside a coil (diameter 30 cm and height 33 cm) (Fig. 1a). The values inside the coil denote magnitudes of the

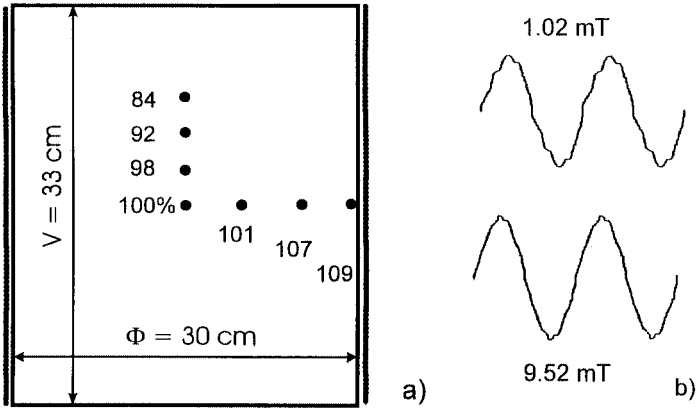


Figure 1. (a) Schematic picture of the coil producing magnetic field. Diameter of the circular cross-section $\Phi = 30$ cm, height $V = 33$ cm. Test tubes are put in the center of the coil (the point denoted by 100%). (b) Wave form of the magnetic field for magnetic induction 1.02 and 9.52 mT.

magnetic field in percent with respect to the center (100%). The test tubes (from green glass Sial) with a suspension of T cells were positioned in the center of the coil. Magnetic field strength (B) was 1.02 and 9.52 mT (denoted as 1 and 10 mT, respectively). Inhomogeneity of the field in the region of the test tube with leukocytes was about 1%. The wave form was sinusoidal, with a small component of higher harmonics, as verified by oscilloscope measurements (Fig. 1b). Exposures to the magnetic field were carried out about 45 min after blood was taken. Exposure time was 60 min (for comparison with standard results of the LAI assay, the exposure time of 60 min was retained). We also investigated longer exposure times (120, 180, 240 min), but no fundamental differences in comparison to 60 min exposure were found. Temperature during the experiment was 37°C.

To elicit the immune reaction, the T cells prepared according to the method described above were mixed with antigen (specific or nonspecific) at a ratio of 1 part antigen to 4 parts T cell float. Antigen was mixed with the suspension just before exposure to the magnetic field. Concentration of antigens was determined as milligrams per milliliter. The same exposure conditions were used for T cells from healthy humans and from cancer patients.

Leukocyte-mediated immunity was assessed by the LAI assay. The number M of nonadherent T cells in suspension was counted after 1 hr of exposure to the magnetic field and without exposure to the magnetic field. The results of the assay were expressed as a nonadherence index (NAI) (5):

$$\mathrm{NAI} = \frac{(M_S - M_N)}{M_N} \times 100$$

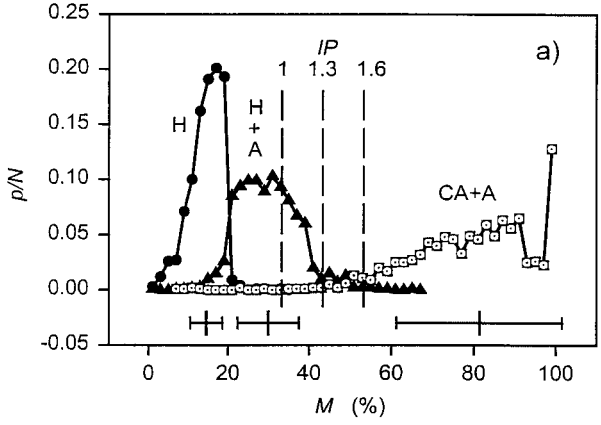
where M_S is the number of nonadherent cells (in percent) in the presence of the specific antigen and M_N is the number of nonadherent cells (in percent) in the presence of the nonspecific antigen (LDV antigen). NAI revealed a difference

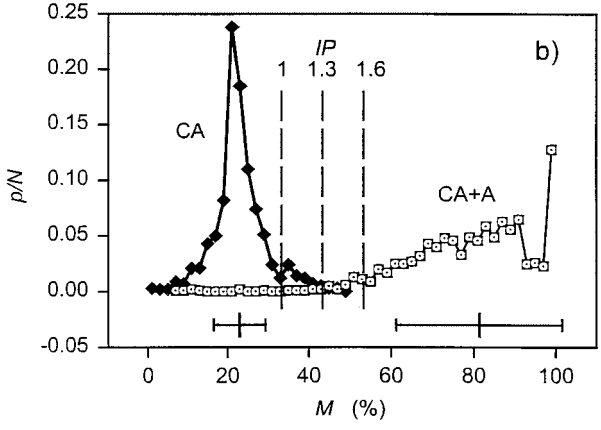
between the action of the specific and the nonspecific antigen, measured in the number of nonadherent cells. Using the NAI value, we cannot distinguish effects of the magnetic field on cells with the specific vs. nonspecific antigen. But we can use only the M values for evaluation of the LAI assay. From comparison of the M values with and without exposure to the magnetic field, we can unequivocally determine its effect.

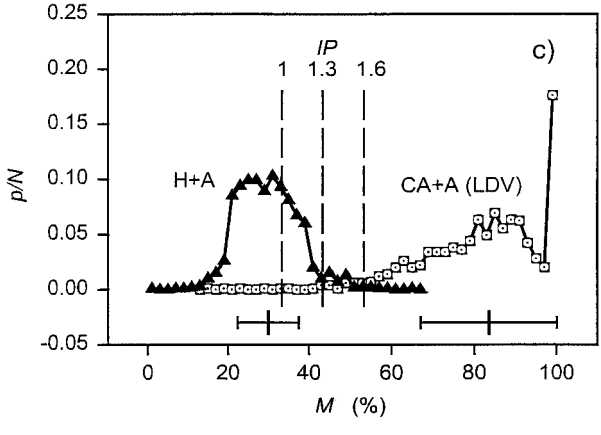
Figure 2 shows the relative frequency p/N of the occurence of the LAI assays whose number of nonadherent lymphocytes is in the region from M to $M + \Delta M$, where p is the frequency of the occurrence, N is the total number of cases, and $\Delta M = 2\%$. Significant differences exist between LAI assay results measured on lymphocytes taken from healthy humans versus those from cancer patients, as illustrated in Fig. 2. To simplify assessment of the LAI assays, we normalized M values by the relation that index of positivity (IP) is equal to M/A, where $A = 100/3$, and determined three discrete levels of IP: 1, 1.3, and 1.6. The majority of the LAI assay cases for lymphocytes taken from cancer patients with specific tumor antigens (CA + A) (Fig. 2a, b) are above IP = 1 (99.4% of cases, i.e., the corresponding probability is 0.994). The probability that the LAI assay of lymphocytes taken from healthy humans with tumor antigens (H + A) (Fig. 1a, c) yields IP $\leq$ 1.6 is also about 0.994. Nevertheless, about 31% of cases were above IP = 1. For practical reasons we used the limits IP = 1.3 and IP = 1.6 to distinguish between IP values of healthy humans and those of cancer patients. A healthy human has IP $<$ 1.3 with the probability of about 0.953. A cancer patient has IP $\leq$ 1.3 and IP $>$ 1.6 with probabilities of 0.989 and 0.956, respectively. Therefore, IP $<$ 1.3 and IP $>$ 1.6 denote states of negative and positive indications for cancer, respectively. The region $1.3 \leq$ IP ≤ 1.6 is a noise region, where the IP values may be suspicious. Nonetheless, various events can shift IP to values higher than 1.3 or 1.6. The p/N versus M for lymphocytes taken from cancer patients with the LDV antigen is illustrated in Figure 2c. The effect of the LDV antigen is similar to that of the specific tumor antigens.

Evaluation of statistical significance was carried out using analysis of variance with repeated measures, the Friedman test, the two-group t-test and a matched t-test.

Figure 2. The relative frequency p/N of the LAI assays as a function of the number M of nonadherent lymphocytes in percent. LAI assays with lymphocytes taken from healthy humans without antigen—H, $N = 1054$ (a) and with tumor antigens—H + A, $N = 1911$ (a, c). LAI assays with lymphocytes taken from cancer patients without antigen—CA, $N = 1304$ (b), with specific tumor antigens—CA + A, $N = 1304$ (a, b), and with LDV antigen—CA + A (LDV), $N = 1373$ (c). The short vertical lines under the p/N curves denote their mean values and standard deviations. The dashed vertical lines denote the principle normalized values of M (i.e., IP = 1, IP = 1.3, and IP = 1.6). The data plotted in this figure represent LAI assay experimental results measured and evaluated by the first author from 1976 to the end of 1992.







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Experimental Results

Figure 3a, b shows mean values and standard deviations of the NAI and the number of nonadherent cells (M) of T cells taken from healthy humans, respectively. Without exposure to the magnetic field, the majority of cells adhere (M = 25.5%) and NAI was less than 5%. The difference in action of both the antigens was small. Exposure to the magnetic field caused an increase of the number of nonadherent cells. M was greater than 50% and NAI was about 10% for exposures at 1 and 10 mT. Exposure to the magnetic field had an inhibitory effect on adherence of T cells in the study. The difference between the mean values of M (or of NAI) with and without exposure to the magnetic field was statistically significant

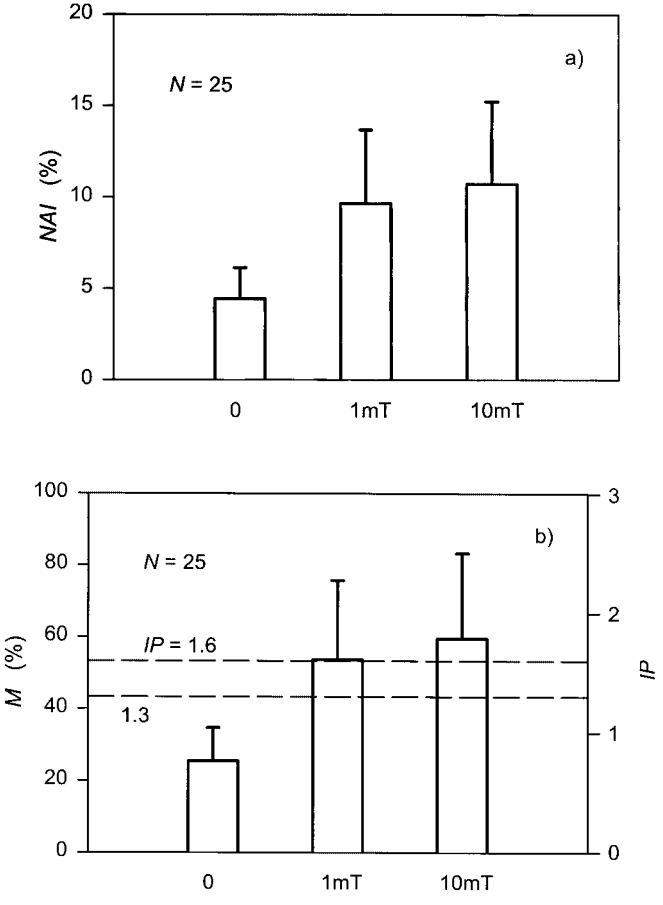


Figure 3. Magnetic field effects on adherence of T lymphocytes taken from healthy humans (N = 25). Ordinate—mean value of the NAI in percent (a) and of the number of nonadherent T lymphocytes (M) in percent (b). Normalized value of the number of nonadherent cells M (i.e., IP) is plotted on the right-hand side vertical axis. The dashed lines denote IP = 1.3 and IP = 1.6. Measured for magnetic induction B = 0, 1, and 10 mT (abscissa). Exposure to the magnetic field increases the number of nonadhering T cells and the NAI value.

at $p < 0.01$ (1 mT) and $p \leq 0.0001$ (10 mT). The difference between mean values of M (or of NAI) for exposure at 1 and 10 mT was not statistically significant.

The mean value of NAI of T cells taken from cancer patients before any medical treatment without exposure to the magnetic field was greater than 15% (Fig. 4a). The majority of T cells (nearly 90%) did not adhere (Fig. 4b). Exposure to the magnetic field diminished the M as well as the NAI values. M decreased to about 55% and NAI to about 10% for 1 and 10 mT. In contrast with the effect of the magnetic field on T cells taken from healthy humans, exposure to the magnetic field had stimulating effect on adherence of the cells under study. The difference between the mean values of M (or of NAI) with and without exposure to

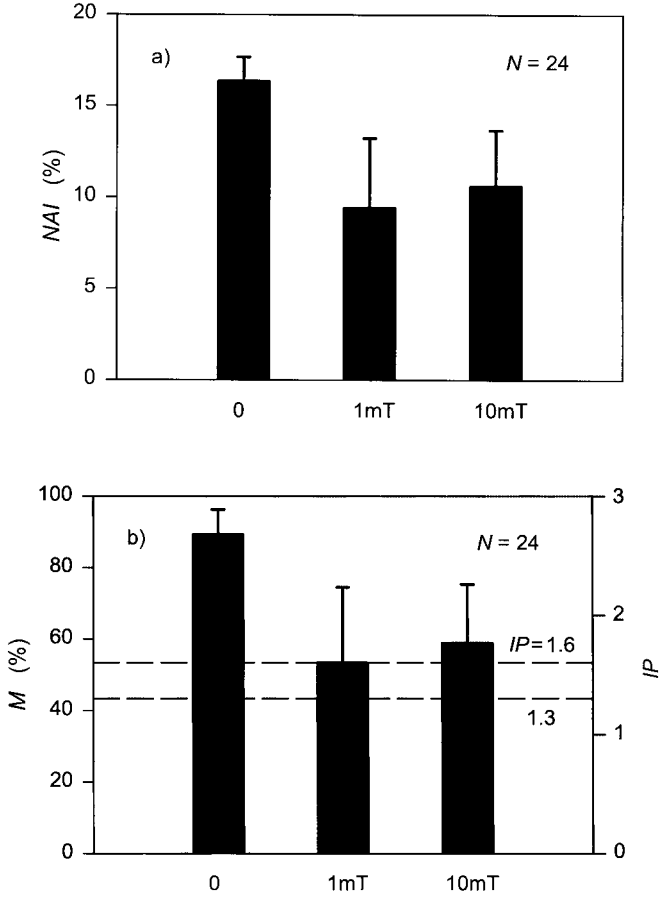


Figure 4. Magnetic field effects on adherence of T lymphocytes taken from cancer patients before any medical treatment ($N = 24$). Ordinate—mean value of the NAI in percent (a) and of the number of nonadherent T lymphocytes (M) in percent (b). Normalized value of the number of nonadherent cells M (i.e., IP) is plotted on the right-hand side vertical axis. The dashed lines denote IP = 1.3 and IP = 1.6. Measured for magnetic induction $B = 0$, 1, and 10 mT (abscissa). Exposure to the magnetic field decreases the number of nonadhering T cells and the NAI value.

the magnetic field was statistically significant at $p < 0.01$ (1 mT) and $p < 0.0001$ (10 mT). The difference between mean values of M (or of NAI) for exposure at 1 and 10 mT was not statistically significant.

The majority of T cells taken from healthy humans without exposure to the magnetic field adhered and the majority of T cells taken from cancer patients did not adhere. Statistical inference that in this case the mean values of NAI are different was $p < 0.0001$. Exposure to the magnetic field acted in opposite ways on T cells from healthy humans and from cancer patients. After exposure to the magnetic field, the mean value of M (or of NAI) of T cells from healthy humans increased and that of T cells from cancer patients decreased.

DISCUSSION

T cell function in the immune system is of the utmost importance. The mechanism of their activation is a complicated process of many steps at different levels in the cell. The chain of activation processes which results in proliferation and secretion of interleukins is still not completely understood. A magnetic field alters some fundamental property necessary for immune function of activated leukocytes. We would like to find the causal mechanism of the magnetic field effects. Because our knowledge of the activation process is not complete, we cannot follow the whole chain of the mechanism of activation and analyze particular steps. Nevertheless, we can perform essential assessment of the interaction mechanism of the magnetic fields with living matter.

Changes in cells caused by magnetic fields seem to depend on mechanisms at two levels. The initial physical transduction step that is fundamental for the effect is followed by stimulated cellular and biochemical pathways. We will give an overview of the physical mechanisms that we propose account for the initial interactions with living cells. Magnetic fields act on magnetic moments and adjust their orientations. Free radical reactions, where unpaired electrons with magnetic moments are ''aligned'' in a magnetic field, are affected if the field is stronger than about 1 mT. Small magnetic particles at the membrane subjected to an oscillating magnetic field may induce pores in the membrane. A magnetic field acts on moving charges (electric currents) by Lorentz force. Electron transfer in an enzyme may be altered, and consequently also the enzyme activity. The field-induced changes in enzyme activity are proportional to charge flow, as have been demonstrated in Na,K ATPase and cytochrome oxidase reactions (37). Polar vibrations in protein structures such as microtubules might be modified. Theoretical analysis predicts that static and oscillating magnetic fields have an essential effect on quantum states of ions within protein cavities and on the dissociation probability of ion–protein complexes (52–56). Nuclear spin of the ion and a nonlinear protein response on redistribution of the ion probability density are involved in the final effect of the magnetic field (52). AC magnetic fields and the Earth's DC field might cause a type of cyclotron resonance of ions. But the experimental evidence

for the theoretical resonance models is still insufficient. Such oscillating magnetic fields induce eddy currents (i.e., circular motion of free charges) due to Faraday induction.

The cellular pathway following the initial interaction has a wide range of possibilities. Magnetic field was believed to interact initially at the level of the cell membrane. Observation of damage to the cell membrane of lymphocytes after exposure to a magnetic field (51) supports this mechanism. The initial interaction must activate a signal transduction pathway that leads inside the cell and to the nucleus. Actually, low frequency magnetic field penetrates the whole cell, and simultaneous interactions can occur anywhere in the cell. Therefore, interaction of leukocytes with the magnetic field may occur at the level of plasma membrane as well as at the level of macromolecules and molecular structures inside the cell. The magnetic field may change the function of ion channels and receptors in the plasma membrane. Inside the cell, many auxiliary enzymes act in the chain of activation of the immune response by T cells. Some of them help in the pathway of hormonal reception. Enzymes contain metal atoms (such as Mg), and an external magnetic field can interact with them and alter enzyme function. It is known that stress proteins (in particular Hsp70) are involved in the immune response to infection and neoplasia (57,58). Because a stress response is elicited after 20 min of exposure to a magnetic field, this mechanism might be important, too. (Gamma-delta T cells respond to heat shock proteins, but their number in the pool is very small. Reaction of these T cells is not the only effect of heat shock proteins in induction of the immune response.)

Altered adherence is a measurable final effect of exposure to magnetic field. The short-range mechanism of adherence depends on adherence molecules in the plasma membrane. The adherence function of adhesive molecules in the membrane (β_2 integrins, e.g., LFA-1, receptor CD2, CD31, L-selectin, E-selectin, etc.) might be altered, e.g., due to ion dissociation (52). If the long-range mechanism of adherence is influenced by a magnetic field, then the London dispersion force has to be considerably diminished, or effectively screened from the substrate (e.g., by a proteinaceous microexudate released from the cell). The same consideration should be true for forces produced by the electromagnetic field generated by excited vibrations in polar structures.

Our present knowledge of the mechanisms of activation and function of T cells does not enable us to determine with confidence which link or links are affected by exposure to the magnetic field.

CONCLUSIONS

We measured the in vitro effects of sinusoidal magnetic field at a frequency of 50 Hz on leukocytes, using the LAI assay. The majority of T lymphocytes taken from healthy humans normally adhere to a substrate, and the NAI value is small. Exposure to a magnetic field increases the number of nonadherent T

lymphocytes, as well as the NAI value. Absent exposure to a magnetic field, the majority of leukocytes taken from cancer patients before any other treatment do not adhere to a substrate, and the NAI value is high. Exposure of such leukocytes to a magnetic field decreases the number of nonadherent T lymphocytes, and the NAI value decreases as well. If the leukocyte adherence inhibition assay is accepted as a measure of cell-mediated immunity, we can conclude that a sinusoidal magnetic field at a frequency of 50 Hz and 1 or 10 mT alters leukocyte-mediated immunity.

ACKNOWLEDGMENTS

This work was supported in part under Grant No. 102/97/0867 of the Grant Agency of the Czech Republic, and under Grant OC 244B.40 of Ministry of Education, Youth, and Sports of the Czech Republic in the framework of COST 244bis. We gratefully acknowledge valuable comments of Professor L. Prokešová and the expert technical assistance of Jitka Švihálková and of Olga Pazderová from the National Institute of Public Health.

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